Pediat. Res. *3:* 269–274 (1969)

Glycine hyperglycinemia
glyoxylate serine

Metabolism of Glyoxylate in Nonketotic Hyperglycinemia

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Extract

Hyperglycinemia is an error of amino acid metabolism in which there are increased amounts of glycine and normal amounts of other amino acids in blood, urine, and cerebrospinal fluid. There are at least two different types of hyperglycinemia. We recently reported on the metabolism of glycine in nonketotic hyperglycinemia [1]. A defect was found in the formation of $^{14}CO_2$ from glycine-1-^{14}C and in the conversion of the carbon 2 of glycine to the carbon 3 of serine. These findings were consistent with a defect in an enzyme system catalyzing the formation of CO_2 and hydroxymethyltetrahydrofolate from glycine, but did not exclude a defect in glycine oxidase. It was decided, therefore, to assess the metabolism of glyoxylate in nonketotic hyperglycinemia.

The conversion of glyoxylate-1-^{14}C to $^{14}CO_2$ (fig. 1) was similar to that of glycine-1-^{14}C. Oxidation to CO_2 was rapid in control subjects, but in the patient, a flat curve was obtained. The transfer of isotope to serine was slower in the patient than in the control subjects. No isotope was found in carbon 3 of serine in the patient, while a significant amount of the isotope of carbon 3 of glyoxylate was incorporated into carbon 3 of serine in the control subjects.

The data obtained indicate that the pathway of preference for the metabolism of glyoxylate is transamination to glycine and rule out a defect in glycine oxidase in nonketotic hyperglycinemia.

Speculation

The data obtained in this investigation contribute to the hypothesis that the enzymic defect in nonketotic hyperglycinemia is situated in the reaction that forms CO_2, NH_3 and FH_4CH_2OH from glycine. This reaction is probably catalyzed by an enzyme system with a number of component enzymes. Further investigations will be necessary in order to determine exactly the enzyme that is deficient.

Introduction

Hyperglycinemia is an error of amino acid metabolism characterized in affected subjects by the presence of increased amounts of glycine in blood, but normal amounts of other amino acids in blood, urine, and cerebrospinal fluid. There are at least two different types of hyperglycinemia, which appear to represent distinct clinical pictures and, probably, different enzymatic defects. CHILDS *et al.* [2] described a patient with ketotic hyperglycinemia who had mental deficiency, neutropenia, and recurrent episodes of ketoacidosis progressing to coma. There have been additional reports of patients with a similar syndrome who usually died at a very early age [9]. GERRITSEN *et al.* [4] described a severely mentally retarded 5 ½-year-old

boy who had hyperglycinemia but lacked most of the manifestations of the ketotic type. He had convulsions and spasticity and, at that time, a strongly decreased excretion of oxalate in urine. For this reason, it was proposed that the metabolic error was at the site of glycine oxidase. This nonketotic type of hyperglycinemia was recently observed in a second patient [17], and the patients reported by MABRY and KARAM [8] and by RAMPINI *et al.* [10] appear now to fall into the same group.

In a study on the metabolism of glycine in nonketotic hyperglycinemia, a defect in the formation of $^{14}CO_2$ from glycine-1-^{14}C was reported [1]. The conversion of glycine-2-^{14}C to serine was considerably slower in patients than in controls. In patients, the conversion of the carbon 2 of glycine to carbon 3 of serine was virtually zero. These findings were considered to be consistent with a defect in an enzyme system catalyzing the formation of CO_2 and hydroxymethyltetrahydrofolate from glycine.

Since a defect in glycine oxidase had not been excluded, an assessment of the metabolism of glyoxylate in nonketotic hyperglycinemia seemed warranted. If the defect were in glycine oxidase, then the metabolism of glyoxylate, its product, should be normal. It was found in the present study that conversions of glyoxylate-1-^{14}C and glyoxylate-2-^{14}C to CO_2 and to carbon 3 of serine, respectively, were quite similar to those of glycine in both the patient and the control subjects. The data indicate that glyoxylate is normally converted to glycine and that the further metabolism of glycine to CO_2 and hydroxymethyltetrahydrofolate is defective in nonketotic hyperglycinemia.

Materials and Methods

Subjects

The patient, S.F., had nonketotic hyperglycinemia and was the subject of the initial report by GERRITSEN *et al.* [4]. At the time of the present study, the child was 8 ½ years old and weighed 18.2 kg. The control subjects were J.N., a retarded 8 ½-year-old male who had brain damage of unknown origin and weighed 16.2 kg, and L.E., a severely retarded microcephalic 5 ¼-year-old female who weighed 14.4 kg.

Isotopic Glyoxylate

Glyoxylate-1-^{14}C (specific activity 8.44 mc/mM) and glyoxylate-2-^{14}C (specific activity 16.0 mc/mM) were obtained from the Nuclear Chicago Corporation. Samples for injection were prepared with isotonic saline solution and sterilized by autoclaving.

Procedures and Analytical Methods

Labeled compounds were injected in amounts of 2 μc/kg of body weight. Collection of expired air and other samples, determination of the isotope content of the CO_2 of expired air, the concentrations and the specific radioactivities of glycine and serine, and the labeling of carbon 3 of serine were performed as described previously [1]. The oxalate content of urine was determined according to the colorimetric procedure of HODGKINSON and ZAREMBSKI [7]. Glyoxylate was determined by the fluorimetric micro method of ZAREMBSKI and HODGKINSON [16].

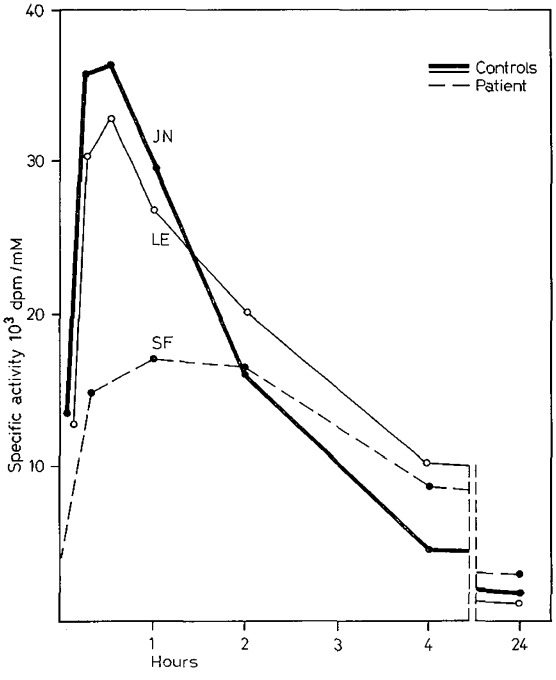
Results

Oxidation of Glyoxylate to CO_2

The conversion of glyoxylate-1-^{14}C to $^{14}CO_2$ is demonstrated in figure 1. The curves closely resemble those obtained after injection of glycine-1-^{14}C. In the control subjects, specific activity (SA) of $^{14}CO_2$ isolated from the expired air reached peak values at 15 to 30 minutes after injection of glyoxylate-1-^{14}C, as compared with values reached 10 to 15 minutes after injection of glycine-1-^{14}C. After reaching the peaks, SA declined in almost linear fashion for four hours. In patient S.F., a rather flat curve was obtained. Maximal height was less than half the highest values of those of

Table I. Isotope content of glycine, serine and carbon 3 of serine in plasma, after the injection of glyoxylate-2-^{14}C

Subject	Time	Glycine			Serine			C-3 of serine
	(min)	dpm/ml	μM/l	dpm/μM	dpm/ml	μM/l	dpm/μM	dpm/μM
Control L. E.	5	533	190	2900	99	94	1050	127
	16	836	197	4250	178	98	1820	145
	31	881	152	5340	340	129	2640	229
Patient S.F.	6	369	667	550	tr	112	–	–
	16	995	620	1600	67	84	798	0
	36	1205	542	2220	90	94	960	0



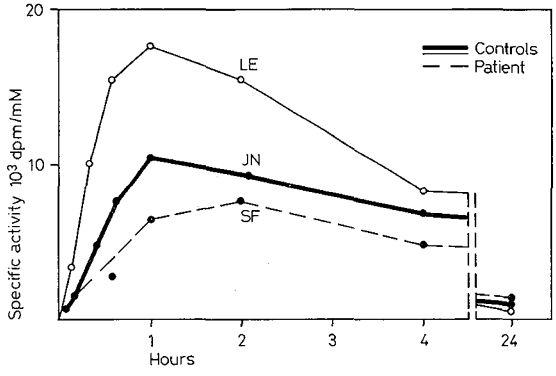


Fig. 2. Specific activities of CO_2 after injection of glyoxylate-2-^{14}C.

◀ *Fig. 1.* Specific activities of expired CO_2 after injection of glyoxylate-1-^{14}C.

Fig. 3. Pathways of metabolism of glyoxylate. Abbreviations employed include FH_4 for tetrahydrofolic acid, CoA for coenzyme A and α-KG for α-ketoglutaric acid. ▼

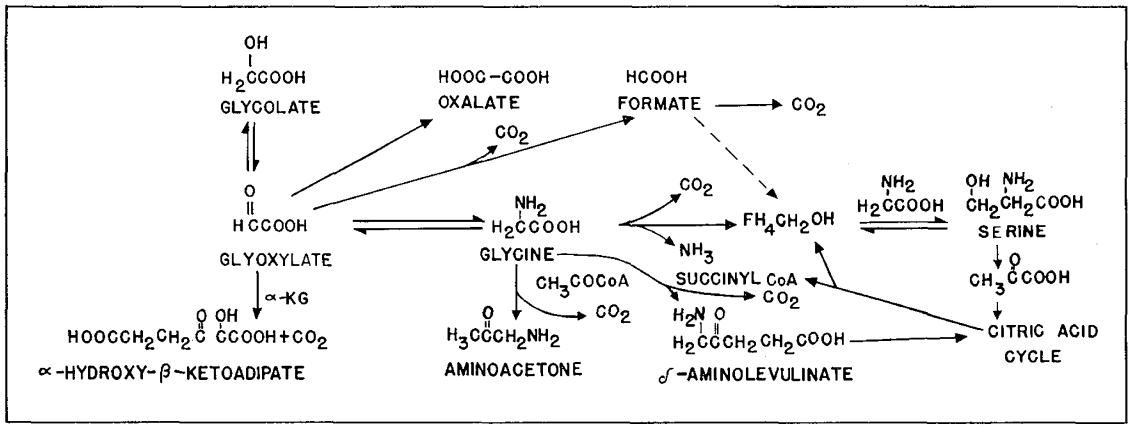


Table II. Excretion of oxalate and glyoxylate in urine

Subject	Glyoxylate (mg/24 h)		Oxalate (mg/24 h)	
	Average	Range	Average	Range
Patient S. F.				
1968	(8)[1] 5.7	0.9–10.7	(4) 15.9	12.5–17.4
1964 [4][2]			(5) 1.7	0.4– 2.4
Controls[3]				
Present study	(12) 1.8	0.9– 3.0	(12) 13.3	2.8–31.6
Reported by others [8]		1.4– 4.7		

[1] Numbers in parentheses refer to the number of determinations.
[2] Eight separate samples were analyzed on the patient.
[3] Single samples were analyzed on 12 control individuals.

the control subjects and was achieved between twenty minutes and two hours. The pattern of conversion of glyoxylate-2-^{14}C to $^{14}CO_2$ was similar in control subjects and in the patient (fig. 2). Maximum values for SA were lower in the patient than in the control subjects, and occurred later. These data, also, were similar to those previously obtained using labeled glycine [1].

Conversion of Glyoxylate to Glycine and Serine

The SA of glycine, serine, and carbon 3 of serine in plasma, drawn after the injection of glyoxylate-2-^{14}C, is shown in table I. The total amounts of isotope (dpm/ml) found in glycine in the plasma of the patient and of the control were similar. The amount was somewhat higher in the patient at the later time points, a finding consistent with inefficient metabolism of the glycine formed. The concentrations of glycine in plasma, however, were much higher in the patient, and SA was lower. The transfer of isotope to serine was slower in the patient than in the control and SA was lower. The data suggest that glyoxylate must be converted to glycine in order to be converted to serine. The SA of serine largely reflects dilution of isotope in the glycine pool, since the ratios of the SA of serine to glycine were found to be practically identical. No isotope was found in carbon 3 of serine in patient S.F., while a significant amount of the isotope of carbon 2 of glyoxylate was incorporated into carbon 3 of serine in the control.

Urinary Excretion of Oxalate and Glyoxylate

Levels of glyoxylate and oxalate in urine from patient S.F., collected recently and remotely [4], were compared with each other and with those of controls (table II). The data obtained recently indicate that excretion of both glyoxylate and oxalate was normal. The mean rate of excretion of glyoxylate was somewhat higher in the patient, but the range was broad. The previously reported levels of excretion of oxalate by patient S.F. were abnormally low [4].

Discussion

Recent studies from these laboratories indicate that in nonketotic hyperglycinemia, there is a defect in the conversion of carbon 1 of glycine to CO_2 and of carbon 2 of glycine to carbon 3 of serine [1]. These data are consistent with the hypothesis that the syndrome represents a genetically induced abnormality in an enzyme system catalyzing the conversion of glycine to CO_2, NH_3, and hydroxymethyltetrahydrofolate [12]; however, a defect in glycine oxidase [4], which catalyzes the deamination of glycine to form glyoxylate, could not be excluded. If glycine oxidase were the site of the block in nonketotic hyperglycinemia, the metabolism of glyoxylate would be expected to be similar in both patients and control subjects. Furthermore, it would be expected that the carbon 2 of glyoxylate would be a highly efficient source of the carbon 3 of serine and that this conversion would be unaffected in nonketotic hyperglycinemia. The data obtained in the present study indicate that the pathway of preference for the metabolism of glyoxylate is transamination to glycine and rule out a defect in glycine oxidase in nonketotic hyperglycinemia.

Metabolic pathways available to glyoxylate are shown in figure 3. The possibility that carbon-1-tetrahydrofolate derivatives may be formed from glyoxylate has been suggested [3, 14, 15]. Ho [6] has studied an enzyme system from chicken liver that catalyzes a reaction between glyoxylate and FH_4 to form N^5N^{10}-carboxymethylene-tetrahydrofolate. DEAN *et al.* [3], however, found that following incubation *in vitro* of human liver or kidney with glyoxylate-1-^{14}C, significant labeling was found only in CO_2 and glycine. Formation of 'active formaldehyde' or hydroxymethyltetrahydrofolate may follow two paths, both involving the formation of formate:

1. $CHOCOOH \rightarrow CO_2 + HCOOH$
2. $CHOCOOH \rightarrow HOOCCOOH \rightarrow HCOOH + CO_2$

In either reaction, formate could be converted to hydroxymethyltetrahydrofolic acid in the following reaction:

3. $HCOOH + ATP + FH_4 \rightarrow \rightarrow \rightarrow FH_4CH_2OH$

Recent investigations on hyperoxaluria [3, 13] indicate that oxalate is an end product, not an intermediate one. Therefore, reaction No. 2 is unlikely.

Another sequence of reactions leading to the formation of CO_2 and an activated 1 carbon product from glyoxylate involves transamination of glyoxylate to glycine prior to decarboxylation as follows:

4. $CHOCOOH + RCH_2NH_2COOH \rightleftharpoons NH_2CH_2COOH + RCOCOOH$
5. $NH_2CH_2COOH + FH_4 \rightarrow CO_2 + NH_3 + FH_4CH_2OH$

Transamination of glyoxylate has been reported to be highly active in the liver of seven different animals using alanine or glutamic acid as amino group donor [11]. The equilibrium favored glycine formation. Our observations suggest that the direct formation of FH_4CH_2OH is not an important pathway for the carbon 2 of glyoxylate. It also appears that glyoxylate is converted first to glycine, which then serves as a precursor of hydroxymethyltetrahydrofolate. Rapid formation of glycine from glyoxylate is also consistent with the findings of SMITH and WILLIAMS [13].

The formation of $^{14}CO_2$ from glyoxylate-1-^{14}C in the patient was clearly less than that in controls. The data are consistent with a defect in reaction 5, the conclusion

of our previous study [1]. It also seemed possible that in reaction 4, the transamination of glyoxylate to glycine might be slower in the presence of a large glycine pool. In experiments with rats [5], increases in the glycine pool achieved by the administration of a large amount of nonisotopic glycine did not change the pattern of oxidation of glyoxylate-1-^{14}C to $^{14}CO_2$, although it did diminish the peak level of SA. Examination of the SA of glycine in the urine of these rats, as well as the percentage of total isotope excreted as glycine, indicated that transamination to glycine is a major metabolic pathway for glyoxylate and is suppressed by the presence of large pools of glycine. In the present study, the high SA of both glycine and serine in blood of both the patient and the control after the injection of glyoxylate-2-^{14}C indicates that transamination is quite active in man. A large glycine pool does not diminish oxidation of glycine to CO_2. On the contrary, increasing the pool of glycine accelerates the rate of conversion [1]. The data shown in table II indicate that the pool of glyoxylate is of normal size. Glyoxylate is also metabolized via a reaction involving conversion of α-ketoglutarate to α-hydroxy-β-ketoadipate, with CO_2 arising from the α-ketoglutarate moiety. Glycine may lead to CO_2 formation in the course of the succinyl-CoA-glycine cycle, which leads to the formation of α-δ-amino leuvlinic acid, or in a similar reaction with acetyl CoA, which yields aminoacetone. Whether these reactions occur in humans is unknown.

The absence in the patient of label in carbon 3 of serine after the injection of glyoxylate-2-^{14}C was a significant finding. In our previous study [1], a block was found in the conversion of glycine-2-^{14}C to carbon 3 of serine in patients with nonketotic hyperglycinemia. Serine may be formed from glycine via reaction 5 and the following reaction:

6. $NH_2CH_2COOH + FH_4CH_2OH \rightleftharpoons HOCH_2CHNH_2COOH$

The data obtained with both labeled glycine and labeled glyoxylate could be explained by a defect in reaction 5. SATO *et al.* [12] have reported an enzyme system catalyzing this reaction in rat liver mitochondria.

In table I, the relative proportion in isotope in carbon 3 is shown to amount to about 10 percent of the total serine activity; however, direct conversion from glycine-2-^{14}C should be examined in order to assess the quantitative significance of this pathway [1]. In control subjects with low glycine pools, SA in carbon 3 was 19 and 27 % of the total serine SA, and in the presence of glycine pools comparable with those of the patients, the SA of carbon 3 was 36 % of the total serine SA. These observations indicate the quantitative importance of a defect in this pathway. Such a defect would be likely to lead to increased concentrations of glycine in body fluids.

There is no ready explanation for the recent findings of normal excretion of oxalate by patient S.F., who had hypooxaluria [4]. Analytical procedures used in the earlier study were different from those used in the present study; notwithstanding, levels of excretion in control subjects were similar in both reports and were in good agreement with values reported by others. It is possible that the metabolism of the patient underwent adaptation, but there is no evidence to support this presumption. It seems more likely that oxalate excretion is quite variable in health and disease and that hypooxaluria is not a consistent feature of the disease. These findings contribute further, however, to relinquishment of the hypothesis that there is a glycine oxidase defect in nonketotic hyperglycinemia.

Summary

The metabolism of glyoxylate has been studied in nonketotic hyperglycinemia. Isotope content was determined in CO_2, glycine, serine and carbon 3 of serine after the separate intravenous injections of glyoxylate-1-^{14}C and glyoxylate-2-^{14}C. An abnormality was found in the conversion of carbon 1 to CO_2 and of carbon 2 to carbon 3 of serine. These data indicate that glyoxylate is readily converted to glycine, and that the further metabolism of glycine is abnormal in nonketotic hyperglycinemia. They exclude a defect in glycine oxidase.

References and Notes

1. ANDO, T.; NYHAN, W.L.; GERRITSEN, T.; GONG, L.; HEINER, D.C. and BRAY, P.F.: Metabolism of glycine in the nonketotic form of hyperglycinemia. Pediat. Res. *2:* 254 (1968).
2. CHILDS, B.; NYHAN, W.L.; BORDEN, M.; BARD, L. and COOKE, R.E.: Idiopathic hyperglycinemia and hyperglycinuria; a new disorder of amino acid metabolism. Pediatrics *27:* 522 (1961).
3. DEAN, B.M.; WATTS, R.W.E. and WESTWICK, W.J.: Metabolism of 1-^{14}C glyoxylate, 1-^{14}C glycollate, 1-^{14}C glycine and 2-^{14}C glycine by homogenates of kidney and liver tissue from hypooxaluric and control subjects. Biochem. J. *105:* 701 (1967).
4. GERRITSEN, T.; KAVEGGIA, E. and WAISMAN, H.A.: A new type of idiopathic hyperglycinemia with hypooxaluria. Pediatrics *36:* 882 (1965).
5. GERRITSEN, T. and REHBERG, M.L.: Unpublished data.

6. Ho, P.P.K.: Function of tetrahydrofolate in the metabolism of glyoxylate. Diss. Abstr. *24:* 4379 (1964).
7. HODGKINSON, A. and ZAREMBSKI, P.M.: The determination of oxalic acid in the urine. Biochem. J. *86:* 16 (1961).
8. MABRY, C.C. and KARAM, A.: Idiopathic hyperglycinemia and hyperglycinuria. Sth med. J. *56:* 1444 (1963).
9. NYHAN, W.L.; ANDO, T. and GERRITSEN, T.: Hyperglycinemia; in Amino acid metabolism and genetic variation (ed. NYHAN, W.L.), p.255 (McGraw-Hill, New York 1967).
10. RAMPINI, S.; VISCHER, D.; CURTIUS, H.C.; ANDERS, P.W.; TANCREDI, F.; FRISCHKNECHT, W. und PRADER, A.: Hereditäre Hyperglycinämie. Helv. paediat. Acta *22:* 135 (1967).
11. ROWSELL, E.V.; CARNIE, J.A. and TAKTAK, B.: Glycine metabolism in mammalian liver. Biochem. J. *101:* 42 P (1966).
12. SATO, T.; MOTOKAWA, Y.; KOCHI, H. and KIKUCHI, G.: Glycine synthesis by extracts of acetone powder of rat liver mitochondria. Biochem. Biophys. Res. Commun. *28:* 495 (1967).
13. SMITH, L.H., Jr. and WILLIAMS, H.E.: Hyperoxaluria (glycolic aciduria); in Amino acid metabolism and genetic variation (ed. NYHAN, W.L.), p.239 (McGraw-Hill, New York 1967).
14. WEINHOUSE, S.: The synthesis and degradation of glycine; in Amino acid metabolism (ed. MCELROY, W.D. and GLASS, H.B.), p.638 (Johns Hopkins Press, Baltimore 1955).
15. WHITE, A.; HANDLER, P. and SMITH, E.L.: Principles of biochemistry, 3rd ed., p.543 (McGraw-Hill, New York 1964).
16. ZAREMBSKI, P.M. and HODGKINSON, A.: The fluorimetric microdetermination of glyoxylic acid in blood, urine and bacterial extracts. Biochem. J. *96:* 218 (1965).
17. ZITER, F.A.; HEINER, D.C.; BRAY, P.F.; MADSEN, J.A. and NYHAN, W.L.: The clinical findings in a patient with nonketotic hyperglycinemia. Pediat. Res. *2:* 250 (1968).
18. This investigation was supported by USPHS Grants HD-02609, HD-00341 from the National Institute of Child Health and Human Development, and FR-00261 from the General Clinical Research Branch, National Institutes of Health, and Children's Bureau Grant No. 408.
19. We wish to thank Drs. E. KAVEGGIA, T. SHIMANECK, and J. TOUSSAINT and the nursing staff of the Central Wisconsin Colony and Training School for their help and advice. These investigations were performed in a manner consistent with the rules and regulations governing human experimentation of all of the participating institutions. Informed parental consent was obtained in each instance.
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